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<p align="center">PRESUMPTIVE AND CONFIRMATORY TESTS FOR BIOLOGICAL SUBSTANCES – FORENSIC BIOLOGY SECTION PROCEDURE MANUAL, SECTION II</p>	<p align="center">Issue No: 2</p>
	<p align="center">Effective Date: 10-January-2005</p>
<p>1 DETECTION OF BLOOD</p> <p>1.1 COMBINED PHENOLPHTHALEIN-TETRAMETHYLBENZIDINE (PTMB) TEST (References 1, 2, 3, Appendix A)</p> <p>1.1.1 Safety Considerations</p> <p>1.1.1.1 Phenolphthalin - Caution! Avoid contact and inhalation!</p> <p>1.1.1.2 Potassium hydroxide - Caution! Corrosive! Poisonous!</p> <p>1.1.1.3 Tetramethylbenzidine - Caution! Harmful if swallowed, inhaled or absorbed through skin! Emits toxic fumes under fire conditions!</p> <p>1.1.1.4 Glacial acetic acid - Caution! Corrosive! Flammable!</p> <p>1.1.1.5 Ethanol - Caution! Flammable! Poisonous!</p> <p>1.1.1.6 Oxidized zinc - Caution! Danger of spontaneous combustion if allowed to dry!</p> <p>1.1.2 Materials and Equipment</p> <p>1.1.2.1 Dropper bottles</p> <p>1.1.2.2 Cotton swabs</p> <p>1.1.2.3 Test tubes, microtiter plates, or filter paper</p> <p>1.1.2.4 100 ml graduated cylinder</p> <p>1.1.2.5 Weigh boats or weigh paper</p> <p>1.1.2.6 Balance</p> <p>1.1.2.7 Spatula</p> <p>1.1.2.8 Scissors</p> <p>1.1.2.9 Tweezers</p> <p>1.1.2.10 Magnetic stir plate</p> <p>1.1.3 Stock Solutions</p>	

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<div data-bbox="354 317 824 348" data-label="Section-Header"> <p>1.1.3.1 Phenolphthalin Stock Solution</p> </div> <div data-bbox="467 386 1237 527" data-label="List-Group"> <ul style="list-style-type: none"> • 1 g Phenolphthalin • 25 g Potassium Hydroxide (KOH) • 100 ml Distilled water • The above ingredients are mixed until thoroughly dissolved. </div> <div data-bbox="467 560 699 592" data-label="Section-Header"> <p>1.1.3.1.1 Storage</p> </div> <div data-bbox="613 625 1533 760" data-label="Text"> <p>1.1.3.1.1.1 This colorless solution is stored under refrigeration over fresh granular zinc to keep it in the reduced form. The oxidized zinc in the bottle should not be allowed to dry (see 1.1.1.6 Safety Considerations and 1.1.3.1.3.1 Disposal).</p> </div> <div data-bbox="467 793 716 825" data-label="Section-Header"> <p>1.1.3.1.2 Labeling</p> </div> <div data-bbox="613 858 1533 1026" data-label="Text"> <p>1.1.3.1.2.1 Label the bottle as Phenolphthalin Stock Solution with a lot number (the date of preparation followed by the initials of the person preparing the stock solution). Example: Phenolphthalin Stock Solution Lot Number 100899JD was prepared by Jane Doe on October 8, 1999.</p> </div> <div data-bbox="613 1060 1511 1127" data-label="Text"> <p>1.1.3.1.2.2 There is no expiration date (see 1.1.5.1 Minimum Standards and Controls).</p> </div> <div data-bbox="467 1161 716 1192" data-label="Section-Header"> <p>1.1.3.1.3 Disposal</p> </div> <div data-bbox="613 1226 1511 1360" data-label="Text"> <p>1.1.3.1.3.1 When the reduced phenolphthalin stock solution is depleted, cover the zinc in the bottom of the bottle completely with a solution of potassium hydroxide in distilled water (25 g KOH/100 ml dH₂O).</p> </div> <div data-bbox="613 1394 1539 1562" data-label="Text"> <p>1.1.3.1.3.2 Label the bottle with the contents and “For Disposal” and refrigerate. Notify the Safety Officer (Eastern, Northern, and Western Laboratories) or the Division Safety Coordinator (Central Laboratory) that the zinc is ready to be disposed of in accordance with Division procedures.</p> </div> <div data-bbox="354 1596 993 1627" data-label="Section-Header"> <p>1.1.3.2 Tetramethylbenzidine (TMB) Stock Solution</p> </div> <div data-bbox="467 1665 1140 1772" data-label="List-Group"> <ul style="list-style-type: none"> • 10 mg Tetramethylbenzidine (TMB) • 30 ml Glacial acetic acid • Mix the above ingredients until thoroughly dissolved. </div> <div data-bbox="467 1806 699 1837" data-label="Section-Header"> <p>1.1.3.2.1 Storage</p> </div> <div data-bbox="613 1871 1528 1938" data-label="Text"> <p>1.1.3.2.1.1 The TMB stock solution may be stored under refrigeration or at room temperature.</p> </div>	

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<p>1.1.3.2.2 Labeling</p> <p>1.1.3.2.2.1 Label the bottle as TMB Stock Solution with a lot number (the date of preparation followed by the initials of the person preparing the stock solution). Example: TMB Stock Solution Lot Number 100899JD was prepared by Jane Doe on October 8, 1999.</p> <p>1.1.3.2.2.2 There is no expiration date (see 1.1.5.1 Minimum Standards and Controls).</p> <p>1.1.3.2.3 Disposal</p> <p>1.1.3.2.3.1 Dispose of the TMB stock solution and other materials contaminated with this solution as hazardous waste in accordance with Division procedures.</p> <p>1.1.4 Working Solutions</p> <ul style="list-style-type: none"> • Distilled water • Ethanol • 3% Hydrogen peroxide • 1:5 dilution of phenolphthalin stock solution in distilled water (1 part of the phenolphthalin stock solution diluted with 4 parts of distilled water) • TMB stock solution <p>1.1.4.1 Storage</p> <p>1.1.4.1.1 All bottles of working solutions are stable at room temperature.</p> <p>1.1.4.2 Labeling</p> <p>1.1.4.2.1 Bottles containing working solutions of ethanol and 3% hydrogen peroxide will be labeled with the contents and the lot number.</p> <p>1.1.4.2.2 The bottle containing the 1:5 dilution of phenolphthalin stock solution must be labeled appropriately. If the lot number of the diluted stock solution is recorded in the reagent log book, then the bottle must be labeled with this lot number. If only the neat stock solution is recorded in the reagent log book, then the bottle must be labeled with the lot number of the neat stock solution, the date of the dilution, and the initials of the person making the dilution.</p> <p>1.1.4.2.3 There is no expiration date for the working solutions (see 1.1.5.1 Minimum Standards and Controls).</p>		

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<p>1.1.5 Minimum Standards and Controls</p> <p>1.1.5.1 On the day of use a positive reagent control (known bloodstain) and a negative reagent control (distilled water) must be tested to ensure that the reagents are working properly. The results of this testing must be documented in the case file.</p> <p>1.1.5.2 If either control does not give the expected result, do not proceed with testing evidence samples until the problem has been resolved as demonstrated by testing another set of positive and negative reagent controls and achieving the expected results with both controls.</p> <p>1.1.5.3 If the results of the test are positive, a substrate control (when available) must also be tested, unless the stain is on a cotton swab, and the results of the testing documented in the case file. It is not necessary to test submitted control swabs.</p> <p>NOTE: Caution should be used when testing leather and suede items since the tannins used on these products may cause a positive result in the absence of blood.</p> <p>1.1.6 COMBINED PHENOLPHTHALEIN-TETRAMETHYLBENZIDINE (PTMB) TEST PROCEDURE</p> <p>1.1.6.1 Gently rub a suspected stain with a cotton swab which has been moistened with distilled water or place a small cutting of the stain in a small test tube or microtiter plate, or on filter paper and moisten with distilled water if desired.</p> <p>1.1.6.2 Add one drop of ethanol.</p> <p>1.1.6.3 Add one drop of 1:5 dilution of phenolphthalin (i.e., the working solution of phenolphthalin).</p> <p>1.1.6.4 Add one drop of 3% hydrogen peroxide.</p> <p>1.1.6.5 Note any color change. An immediate pink color is expected if blood is present.</p> <p>1.1.6.6 Add one drop of tetramethylbenzidine stock solution.</p> <p>1.1.6.7 Note any color change. An immediate blue-green color is expected if blood is present.</p> <p>1.1.6.8 Interpretation</p> <p>1.1.6.8.1 Positive Reaction = Immediate pink color at 1.1.6.5, followed by immediate blue-green color at 1.1.6.7</p> <p>1.1.6.8.2 Negative Reaction = No color change at 1.1.6.5, followed by no color change at 1.1.6.7</p> <p>1.1.6.8.3 Inconclusive Reaction = Development of color combinations other than those specified for a positive reaction, including one test positive and the other test negative</p>	

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<p>1.1.6.9 Reporting Results</p> <p>1.1.6.9.1 Report positive test results as “blood was indicated...”</p> <p>1.1.6.9.2 Report negative test results as “no blood was detected...”</p> <p>1.1.6.9.3 Report inconclusive test results as “tests for blood were inconclusive...”</p> <p>1.2 LUMINOL TEST (Reference 4, Appendix A)</p> <p>1.2.1 Safety Considerations</p> <p>1.2.1.1 Sodium perborate - Caution! Harmful if swallowed, inhaled or absorbed through skin!</p> <p>1.2.1.2 Aminophthalhydrazide (luminol) - Caution! Irritant! Emits toxic fumes under fire conditions!</p> <p>1.2.2 Materials and Equipment</p> <p>1.2.2.1 Spray bottle (must contain no metal parts as the luminol reacts with some metals)</p> <p>1.2.2.2 50 ml graduated cylinder</p> <p>1.2.2.3 Balance</p> <p>1.2.2.4 Weigh boats or weigh paper</p> <p>1.2.2.5 Spatula</p> <p>1.2.2.6 Ziploc bags, conical tubes, or other appropriate containers (optional)</p> <p>1.2.2.7 Magnetic stir plate</p> <p>1.2.3 Stock Solutions</p> <p>1.2.3.1 Solution A</p> <ul style="list-style-type: none"> • 0.7 g Sodium perborate • 50.0 ml Distilled water • Mix above ingredients until thoroughly dissolved. • USE IMMEDIATELY! DO NOT STORE. <p>1.2.3.2 Solution B</p> <ul style="list-style-type: none"> • 0.1 g Aminophthalhydrazide (luminol) • 5.0 g Sodium carbonate • 50.0 ml Distilled water • Mix the above ingredients until thoroughly dissolved. 	

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<p style="text-align: center;">• USE IMMEDIATELY! DO NOT STORE.</p> <p>NOTE: The dry chemicals in Solutions A and B can be weighed out and placed in appropriately labeled containers and stored for up to 6 months in the dark at room temperature. Each container must be labeled with the date prepared, the expiration date, the initials of the person who prepared each package, and the amount of distilled water to be added. Water can be added when needed.</p> <p>1.2.4 Minimum Standards and Controls</p> <p>1.2.4.1 Test a positive reagent control (known bloodstain) and a negative reagent control (distilled water) to ensure that the reagents are working properly. The results of this testing must be documented in the case file.</p> <p>1.2.4.2 If either control does not give the expected result, do not proceed with testing evidence samples until the problem has been resolved as demonstrated by testing another set of positive and negative reagent controls and achieving the expected results with both controls.</p> <p>1.2.4.3 If the results of the test are positive, a substrate control must also be tested (when available) and the results of the testing documented in the case file.</p> <p>1.2.5 LUMINOL PROCEDURE</p> <p>1.2.5.1 When the test is ready to be conducted, mix equal parts of solutions A and B and place in a sprayer bottle.</p> <p>1.2.5.2 Under darkened conditions, immediately after mixing equal parts of solutions A and B, spray the positive and negative controls to ensure that the reagents are working properly. If both controls give the expected results, proceed with spraying the area of interest and, as appropriate, a substrate control (when available). Document results in the case file.</p> <p>1.2.5.3 Areas containing blood will luminesce immediately.</p> <p>1.2.5.4 Mark luminescent areas for subsequent testing with the Combined Phenolphthalein-Tetramethylbenzidine Test.</p> <p>1.2.5.5 Interpretation</p> <p>1.2.5.5.1 Positive Reaction = Immediate luminescence</p> <p>1.2.5.5.2 Negative Reaction = No luminescence</p> <p>1.2.5.5.3 Inconclusive Reaction = Slow and/or weak luminescence</p> <p style="text-align: right;">◆END</p>	